SCIENCE

FRIDAY, MARCH 27, 1914

CONTENTS

MSS. intended for publication and books, etc., intended for review should be sent to Professor J. McKeen Cattell, Garrison-on-Hudson, N. Y.

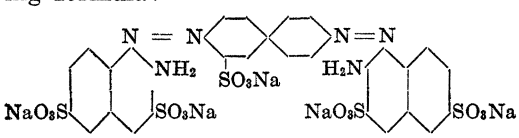
THE ACTION OF VITAL STAINS BELONGING TO THE BENZIDINE GROUP[1]

THE brilliant advances in our knowledge of the chemistry of aniline dyes, brought about naturally by the enormous commercial importance which the dyes possess, has been brought to bear, and will in the future be brought to bear, we believe, in the solution of some important problems in biology. The dyes possess peculiar advantages; especially is this true in the case of those of them which undergo little or no chemical transformation when injected into the living body. To this class of dyes, as we hope to show later, belong the benzidine or substantative dyes. It might be inquired immediately whether vastly more important results could not be secured from the study of dyes which, on the contrary, are known to suffer definite chemical changes within the body, for it might be supposed, for instance, that valuable light could be thrown on oxidative or reductive processes peculiar to certain cells or tissues. It was, of course, with motives not far removed from these, that Ehrlich first seriously attempted the use of dyes to solve the problem of the relation between pharmacological action and chemical constitution in his classical essay on this thesis in 1902. When we insist, however,

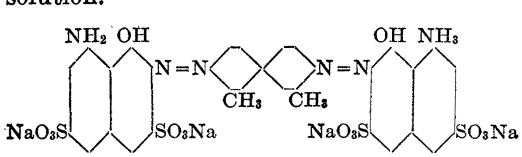
[1] Read at the session of the National Academy of Sciences, Baltimore, November 18, 1913. From the Anatomical Laboratory, Johns Hopkins University and the Kgl. chirurgisches Institut, Breslau. The study is a preliminary report of observations which will be presented in full in the Memoirs of the Rockefeller Institute for Medical Research and which were rendered possible by grants from the Rockefeller Institute and the Robert Koch Stiftung, Berlin.

that the study of dyes which suffer no chemical change within the body is of the highest value and indeed calculated to lead us to results which can be secured in no other way, we do so mainly because the class of dyes to which we refer can be injected in relatively large quantities into the blood stream of living animals without perceptible toxic effect; and the dye, taken care of as it were by definite cells which store it unchanged within their cytoplasm, can be detected without difficulty, wherever it may be, on account of its color.

In 1905, Ehrlich and Shiga, then attempting the cure of trypanosome infections in laboratory animals, happened to find that the azo dye, which they named trypan red and which possesses the following formula:



could be injected in sufficient quantity into the living animal to kill the organisms of the disease without perceptible toxic effect to the cells or tissues of the host, themselves deeply stained. A year later, driven thither in the same quest, Nicolle and Mesnil, of Paris, discovered a similar effective compound or "good color" as they called it in trypan blue, a dye formed by the combination of two molecules of 1.8 amidonaphtol 3.6 disulphonic acid with one molecule of diazotized ortho-tolidine in alkaline solution.



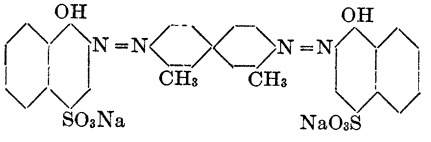
The profound color of the healthy animals which received this dye could not fail to attract the attention of Nicolle and Mesnil, who set Boufard the fascinating problem of determining in what form the dye persisted in the body. His report, which was all too short, nevertheless acquainted us with the main fact that the dye had not merely remained in the fluids of the body or had pervaded the organs and tissues in a profuse way, but was engulfed in the bodies of certain definite cells primarily of one type which we shall have occasion to describe carefully shortly. Coincidently Goldman at Ehrlich's suggestion took up the same theme, and his enthusiastic studies have in spite of occasional inaccuracy attracted general interest to the subject.

Nowhere however in the rapid literature which has begun to accumulate on this subject can one find an attempted answer to the fundamental question of how the dye really acts on the cells of the body, *i. e.*, what property it is by virtue of which one of the members of this class of dyes is enabled to be a brilliant vital stain; whereas a closely related dye is a complete failure. Nor, secondly, does it seem to me that full advantage has been taken of the great opportunity bestowed by these dyes in enabling us to detect a hitherto unknown or unrecognized function of a great mass of cells all over the body which can now be grouped together under a common designation as a great system or tissue.

If we inject into the peritoneal cavity of a mouse 1 c.c. of a one half per cent. solution of trypan blue, we can observe within a few minutes that the ears, the tip of the nose, the tail, the mucous membranes and soon the skin of the entire body have begun to blue, and that this deepens rapidly in intensity, so that within a few hours a maximal deep blue color is possessed by the animal, a color which, in spite of this single dose, is not lost for many weeks. The animal thus stained plays, eats, breeds, and in all ways manifests its normal activity, and

there is no evidence of the ill effect of the dye.

If now instead of trypan blue we employ the brilliant blue dye azo-blue whose constitution is



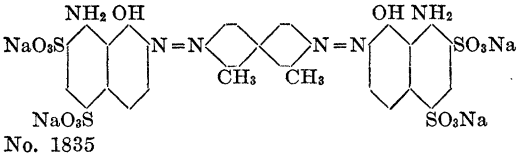
we are met by a striking difference. Neither within minutes or hours after the intraperitoneal injection of such a dye is any trace of color to be seen from the dye, and the repeated injection of the dye over a long period of time does not in any way change this negative result. The autopsy of such an animal shows indeed that we have heaped upon it a large quantity of a colored foreign body, for the dye has remained on the whole where it was injected, that is, in the peritoneum and the structures connected with it. Why this difference?

Several notions have been advanced in explanation of the action of vital stains. Primarily we have to do with the ideas, first, that a chemical union exists between the dye molecules and some portion of the cell protoplasm, and, second, the theory that dyes owe their ability to stain vitally to some physical property by virtue of which they can penetrate the cell. The name of Ehrlich is connected with the first of these two views, while that of Overton is identified with the second, and we may mention here that the commonest physical rather than chemical explanation of vital staining has been that the dyes in question are soluble in fat or fat-like bodies, a layer of which was assumed to envelop the cell. While this explanation may have been convincing in the explanation of vital staining obtained by the earliest known vital stains (methylene blue and neutral red) we are aware now that both of the assumptions involved in it were gratuitous, for neither are the benzidine dyes soluble in fats or lipoids nor are the majority of the cells of the body surrounded by a layer of these substances. If, then, in spite of the above, we must maintain that after all physical factors play a predominant rôle in the vital staining produced by the azo or benzidine dyes, it is for reasons quite apart from those involved in Overton's lipoid theory.

The chemical explanation of a vital stain presupposes that there exists between some element of the protoplasm of the cell, the chemo-receptor in the sense of Ehrlich, and some part of the dye molecule a combination, such as we are accustomed to see take place between two bodies in accordance with the laws of formal valence. It is only necessary to show how important such an explanation is in the mind of Ehrlich by mentioning the fact that he assigns the therapeutic effects of the drug salvarsan primarily to an affinity which he assumes to exist between the parasites of syphilis and a precise part of the salvarsan molecule; namely, the ortho-amido-phenol configuration. The application of this principle in an explanation of the vital stain produced by trypan blue must lead us to maintain that a similar configuration, *i. e.*, the peri-amido-naphtol configuration, is really responsible for the union between dye and cell which gives us the vital stain here. We can dispense with such reasoning shortly.

Evidence which would appear to concern the efficacy of a peri-amido-naphtol ceptor is furnished by the brilliant vital staining produced by the dyes Nos. 1824, 1835 and 1846, in all of which the hydroxyl and amido groups are in a peri position to one another, and the azo bond at position II.

No. 1824



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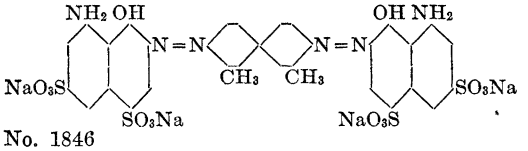

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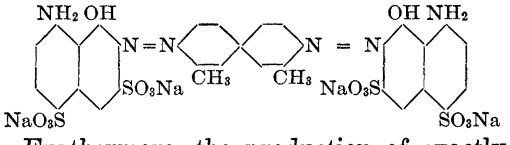
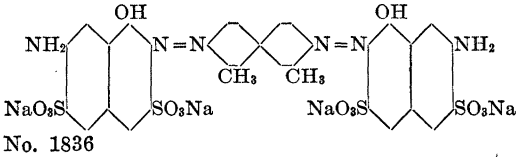


Furthermore, the production of exactly as brilliant a result by dyes in which merely the position of the OH group has remained constant, the NH_2 group either being absent or shifted in its position, might lead us to suppose that it is the alpha position of the hydroxyl radicle rather than the entire peri-amido complex which in all these cases has determined the vital stain. We mention of such dyes the vital stains 2836, 1836 dioxy, 1527, 136, 1368.

No. 2836



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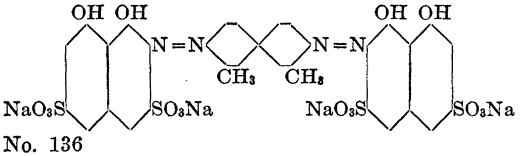
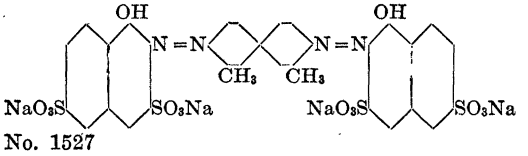
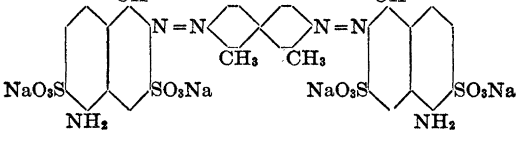


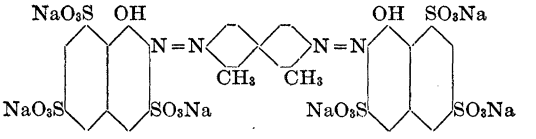
No. 136



No. 1527

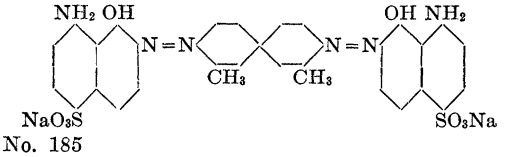


No. 1368



This conclusion, however, (*i. e.*, that an alpha-naphtol receptor has been responsible for the staining) must be rejected at once as soon as we examine the dyes 184, 185, 257, 258, 286, 184 dioxy 14 and 15 all of which can be injected into animals without producing a vital stain.

No. 184



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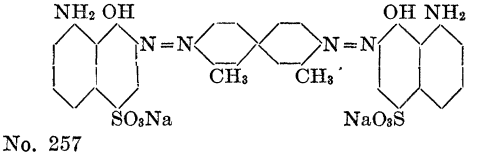

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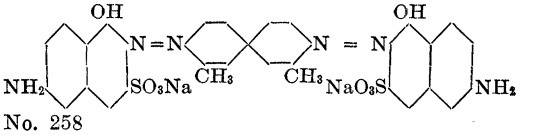

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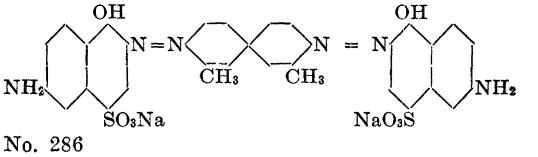

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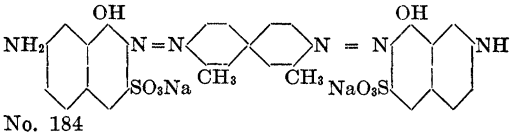

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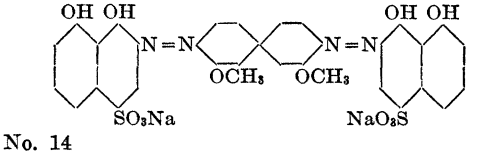
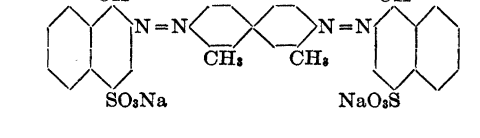
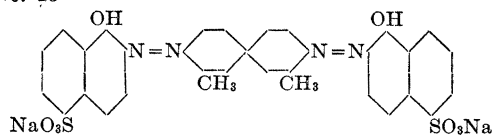


No. 14

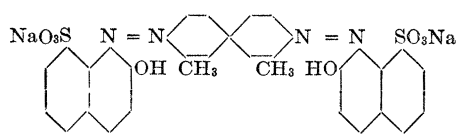


No. 15



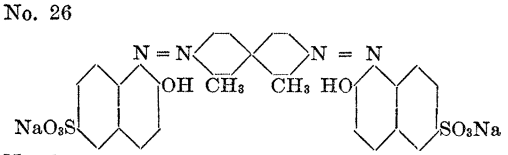
It is apparent then from the foregoing that if the alpha-naphtol receptor is to work, it can only do so in the presence of two sulfonic acid groups.

When, now, we examine the behavior of the beta-naphtol sulfonic acids in this respect, we find that, strangely enough, one of the monosulfonic acids (namely the 2.8 acid) produces a dye which acts as a vital stain.[2]

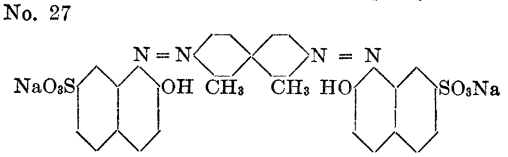


The other mono acids which we have examined (the 2.6 and 2.7 acids) produce entirely negative dyes.

No. 26

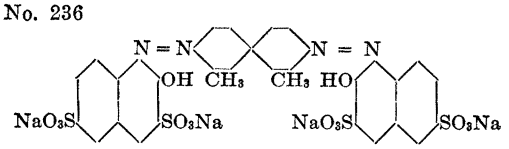


No. 27



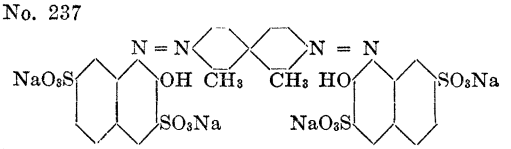
Whereas of the disulfonic acids, the 2.3.7 and 2.6.8 acids produce dyes which are non-stainers, the 2.3.6 acid combined with tolidine furnishes a dye which approaches a true vital stain.

No. 236



[2] The dye was made for us by Dr. Taggesel through the kindness of Schaellkopf, Hartford and Hanna, Buffalo.

No. 237



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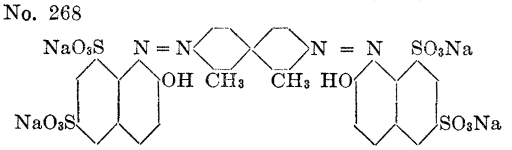
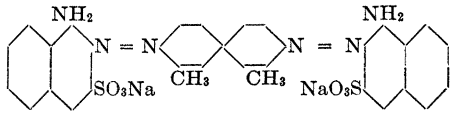


A comparison of the different effects secured by the two dyes 236 and 237 is interesting, for it is difficult to believe that the exact position which the sulfonic acid radicle assumes in the naphthaline ring is an adequate explanation of this. In all the dyes which we have examined, the number of sulfonic groups has been important, but not the position which they occupy. The precise point of insertion in the naphthaline ring was of little moment and we have to inquire in dyes 236 and 237 why the shifting of one SO_3Na group from 6 to 7 changes a positive into a negative dye. Now these two isomeric acids, apart from their close chemical relationship, have long been known to produce dyes which differ greatly in some of their non-chemical characteristics—characteristics which we believe determine whether a dye of the benzidine class shall be a vital stain or not.

For the present, however, let us believe that there is a general alpha-naphtol-disulfonic ceptor, a particular beta-naphtol-disulfonic ceptor (viz., the 236 one) and a particular beta-naphtol-monosulfonic ceptor (the 28 one) whereas none of the other monosulfonic and none of the other beta disulfonic acids have chemoceptors corresponding to them in the cell.

When we consider the dyes made from the naphtylamines we must still further extend our list of hypothetical chemoceptors, for many of these dyes are brilliant vital stains, among them 13, 15, 16, 26, 27 and 28.

No. 13



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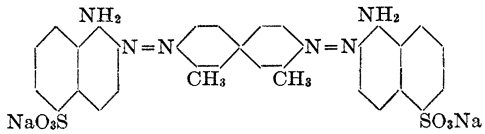

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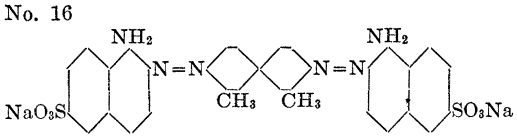

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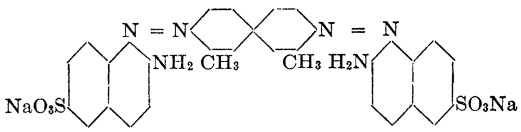

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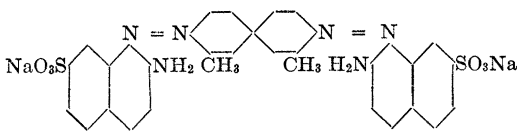
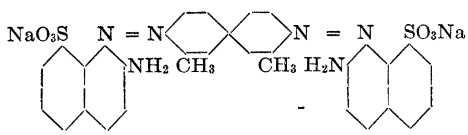
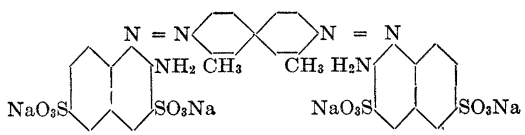


No. 28

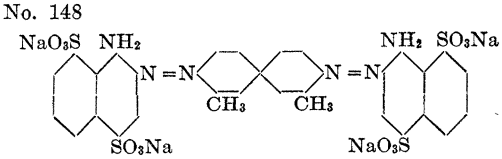


Both of the naphtylamine disulfonic acids with which we have worked have yielded equally brilliant results (the 2.3.6 and 1.4.8 ones).

No. 236

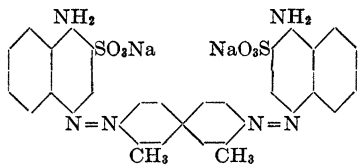


No. 148



We meet, however, among the naphtylamines surprises which are just as remarkable as those which greeted us in the naphtol group, for the isomeric dyes which are listed below (12, 14, 17, 18 and 25) are all negative.

No. 12



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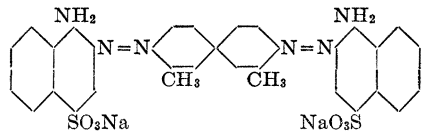

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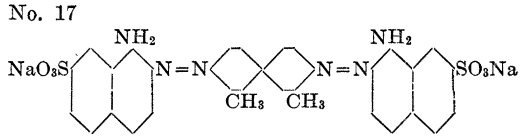

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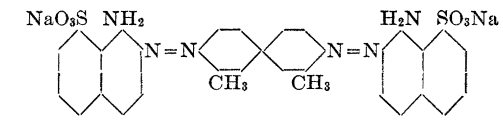
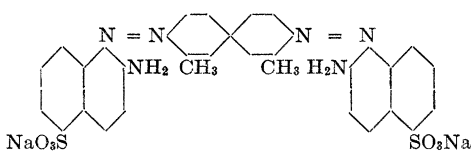
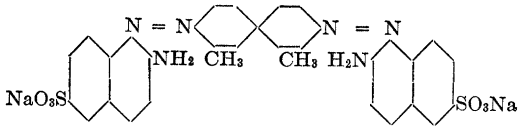


No. 25



We shall not have to insist that it is hardly likely that chemical reaction between these dyes and substances with which they could truly combine would hardly occur or fail to occur in this capricious way. Our results then are hardly capable of being formulated in terms of the chemoceptor theory, for there is no one chemical configuration which we are able to pick as a chemoceptor. Our search at this juncture for some common characteristic possessed by a positive in contrast to negative dye, a characteristic which when possessed always permitted the dye to be a vital stain and when absent led us to predict its failure, was rewarded with success. We were in short induced to look into the phys-

ical state of the dye solutions. Our attention was rather dramatically called to this phase by experiments with benzo-purpurine B:

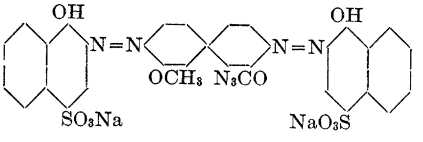


A fresh, cold one-per-cent. solution of this dye can be injected rapidly into the ear vein of the living rabbit and will produce within a few minutes a beautiful diffuse coloring of the whole animal, a coloring which though at first affecting merely the body fluid, can in a few hours be seen to have established itself in the form of granules in that distinct class of cells which are affected by dyes of this group. If, on the other hand, we inject a similar cold solution of this dye, but one which has been allowed to stand a few days before using, the animal invariably dies before our eyes with typical symptoms of cerebral embolism. If we boil the dye for a little while with Ringer's solution instead of with water, the effect is even more marked, for a single cubic centimeter of such a solution after it has been allowed to cool, kills the animal instantly on injection. Why are the dye solutions so different in their behavior? A few test-tube experiments sufficiently answered our question, for we were dealing with an electrolytic precipitation of a colloid, whose coagula were sufficient to plug the cerebral vessels. By testing in this way all of the dyes which we have reported as negative, we were able to show that no one of them failed to produce embolism on injection.

But while thus dangerous in the living blood stream there might still be thought to be no reason why these dyes should fail to act when placed under the skin or in the body cavity. In these situations, however, the dyes remain without invading much of the remainder of the body, and we were led to test the diffusion power of positive and negative dyes in a medium where it was slow enough to be measured, *i. e.*, in 2 per cent. gelatin, and without presenting here our tabular results we found no exception to the rule that positive dyes possessed a rapid diffusion rate, while negative dyes little if any diffusion at all.

These facts harmonize, of course, perfectly with our knowledge of the behavior of colloids, and make it certain that we are dealing with phenomena which depend on the size of the particles or aggregates in our solution. A negative dye could not reach many cells in the body, for its particles are all large and rapidly agglutinated in the body fluids, and such large particles have slight, if any, power of diffusion. Did, however, the cells in their neighborhood accept these negative dyes?

The investigation of the cells of the subcutaneous tissues at the injection site showed that even with the most negative dyes a vital staining in this limited zone had always taken place. Our failure to stain more cells then was due solely to our inability to reach them. We proved this contention, we believe, conclusively by selecting a dye D-14.

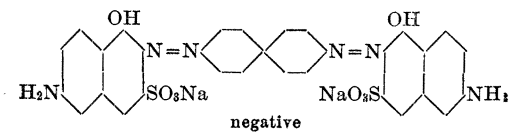


which when placed under the skin has practically no powers of diffusion and which when injected at a normal rate into the blood stream always kills with the typical picture of embolism. We found that the very slow injection of a perfectly fresh, cold one half per cent. solution in distilled water obviated this accident; and although for a few days the continual injection of

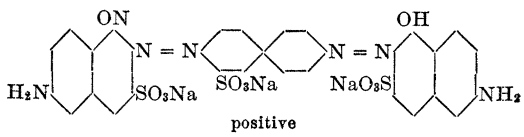
the dye did not color the skin or mucous membranes of the animal, this was eventually obtained. The study of an animal in the early stage of such a series of injections is most interesting. We have indicated that such a negative dye must consist chiefly of large particles, and since little color appears in the urine of these animals, and the animal is externally unstained, it is evident that the dye particles are engulfed by cells internally located. The autopsy of such an animal shows that not only the skin but most of its tissues are free from dye with four notable exceptions; namely the liver, the spleen, the lymph-glands, and the bone marrow, all of which are blue-black.

We do not believe that a heightened power to attract the dye is possessed by the cells in these localities. The skin cells in the neighborhood of a puncture take up the stain almost as rapidly, but they are unable to do it when the large dye particles are only circulating in the blood vessels, from which their lack of diffusion powers never take them, for they can not leak through the walls of the capillaries.

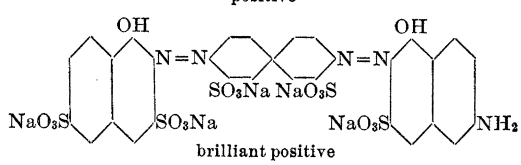
A glance at the list of positive and negative dyes which is just presented shows us that most positive dyes are disulfonic acids, a statement, in view of the ideas which we have just developed, which means that the sulfonic acid radicle exerts a favorable effect on the character of the dye solution, *i. e.*, makes it more soluble, more diffusible, and, so, quickly distributed, that is, *a true vital stain.* Evidence to this effect has already accumulated in a comparison with the positive and negative dyes 184, 185, 1846, 1824 and 1835, but this conclusion is, we think, substantiated in a more complete and overwhelming way by the sulfonation of a number of negative dyes by which means brilliant, positive stains were always secured. We cite as a good example of this



negative

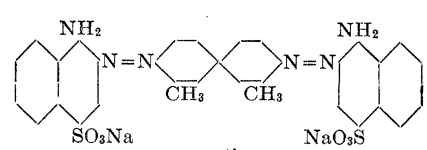


positive

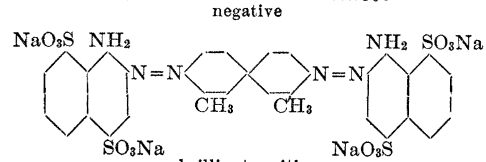


brilliant positive

Identical results were secured with the 2.8 amido naphtol 6 sulfonic acid combined with benzidine, with benzidine monosulfonic acid and with benzidine disulfonic acid; and a similar effect of sulfonation is seen in the brilliant tetra-sulfonic congo red dye made from the 1 naphtylamine 4.8 disulfonic acid, whereas the congo red monosulfonic acid (the 1 naphtylamine 4 sulfonic acid) or naphtionic acid yields a dye which is not a vital stain.



negative



brilliant positive

In this connection the question of whether or not it was possible to over-sulphonate the dye was attempted with interesting results. The sulfonation of diamine blue 2B gives us a dye which while a vital stain nevertheless passes the kidney much more rapidly than the original body, so that after an intense stain the animal is decolorized again in a few days. We have secured identical results in experiments with a similar sulfonated trypan blue.

The staining with acid azo dyes then is a physical phenomenon, and when we turned our attention to the likelihood of other solutions or pseudo-solutions giving an identical picture we are able to say that such is indeed the case, for those excellent suspensions of fine particles of gold, silver, palladium and some other metals which are called colloidal solutions eventually stained an animal just as did our vital dye. This effect has in fact been known for a long time for the so-called argyria, a pigmentation of the mucous membranes of workers concerned with silver salts, is a similar storage of particles formed by the silver when it meets the fluids in the body.

It remains now to discuss briefly what cells or tissues in the body are selectively affected by these stains. We can do this best by describing the autopsy of an animal which has been injected, on two occasions three days apart, with trypan blue and killed the day following the last injection when the animal with every appearance of health is intense blue. The skin of the abdomen when stripped back is seen to be intensely stained, but freshly spread preparations from the subcutaneous tissue show that the color is only to a minor degree due to the coloration of the fluids, but mainly to great numbers of small though dense granular deposits in certain cells. The larger and more striking of the cells possess a large slightly irregular oval nucleus, and an elongated cell body with frequent though always sharply defined pseudopodia or larger cell processes. Irregularly scattered through the cytoplasm are the granular deposits of the dye, and the larger of these are always comprised in a vacuole inside of which both larger and tinier fragments are in liveliest Brownian movement. We have identified these large, brilliantly stained cells of the skin and connective tissue of the body as the cells which have been designated variously by histologists as the resting wandering cells (Maximow); the clasmatocytes (Ranvier), the adventitia cells (Marchand) and the rhagiocrine cells (Regaud). At the same time in smaller, although more abundant cells, cells with more regularly oval nuclei and with faintly defined nuclear membrane and cytoplasmic boundaries, in these cells are fine scattered granules of the stain. These are, we believe, the most abundant and typical cells of connective tissue and are probably those designated as fibroblasts by the majority of investigators. None of the blood cells nor the endothelial cells of the capillaries of the skin carry the minutest trace of the dye. The muscles of the body, instead of their customary pink hue, are greenish in color, due to the infiltration in the minute connective tissue septa of countless numbers of these vitally stained cells of both types. The peritoneal cavity contains a normal amount of fluid which is pale blue in color and in which are suspended many vitally stained cells. The most brilliant of these and the largest are those cells which have been designated by Mechnikoff, Schott and others as makrophages—cells which, spherical in shape, are covered with delicate pseudopodia, and the cytoplasm of which is often a frothy mass of vacuoles, in which are always particles of the dye. We can trace all transition stages from these cells to those but slightly larger than lymphocytes, all of them containing some inclusions of the vital stain.

The cells which line the peritoneum, the mesothelial cells of Minot, possess in a peripheral zone the fine granulations which are characteristic of the cells of type 2 in the skin.

Of the peritoneal organs the liver and kidney are undoubtedly the deepest stained, for they are almost a blue black, a color due in the liver almost exclusively to the inclu-

sions of the dye by the cells of Kupfer of the hepatic capillaries and in the kidney to a similar behavior shown by the epithelial cells of the proximal convoluted tubules. The spleen and lymph glands are stained a paler though still brilliant blue and the color is due in these organs to the granules carried by the so-called reticular and by the true lymphatic endothelial cells.

In the testis the interstitial cells of Leydig are stained brilliantly, although the granules here are singularly regular and intermediate in size between those possessed by cells of type one and two in the skin. In addition, true vitally stained connective tissue cells of type two are also present between the seminiferous ducts, but the epithelial cells of the seminiferous tubules never contain the slightest trace of the dye.

The pancreas possesses in the connective tissue septa of its acini brilliantly stained cells of type one, although no particle of the dye is found in the pancreatic parenchyma.

The thoracic cavity presents a remarkable picture, for its deep blue walls enclose the lungs which have preserved their normal pink color, and in which few vitally stained cells are found. The heart, however, contains many of these, for not only do the epicardial cells receive the dye like the mesothelial ones, but also many connective tissue cells of type one infiltrate its musculature at many points.

The thymus, thyroid and parathyroids owe their generally lighter stain to similar cells which follow the connective tissue in its structural relation to these organs.

Most remarkable is the behavior of the central nervous system, for with the exception of the hypophysis and choroid plexus the nervous system contains no trace whatever of the dye, and its whiteness in contrast to the stain of the other tissues is a strange sight, the more so because the dura is always densely stained.

With the exception of the kidney, the epithelium of whose convoluted tubules comes from the middle germ layer, the great mass of the epithelium of the body refuses to react to the stain. Most remarkable also is the rejection of the dye by the blood cells, whose polymorphonuclear elements have always been supposed to play so great a rôle in phagocytizing foreign particles.

It might possibly be supposed from the comparative physical measurements we have made on the dyes that the so-called vital stains or vital granules, which we have just described, are an expression merely of diffusion, especially inasmuch as the most diffusible dyes of this class are the most satisfactory vital stains; but we have explicitly pointed out that heightened diffusion powers effect the vital stain merely by enabling the dye molecules or dye-molecular-complexes to present themselves to the class of cells which will receive them in every corner of the body. Having arrived at the cell, the dye by no means diffuses into it, as we can see, for instance, various basic dyes (neutral red, methylene blue, janus green) diffuse into a cell, advancing from periphery to center, and lodging often with special avidity in some preformed granule or structural element of the cell (*e. g.*, mast cell granules with thionin or neutral red, Nissl bodies with methylene blue). The benzidine dyes, let in more slowly at the cell's periphery, never encounter physical conditions which favor their rapid spread and they are disposed of by the cell by being concentrated at various points where they are doubtless set apart from the cell's protoplasm. The vital granules then deserve their designation, for they are always the result of the behavior toward the dye on the part of a living cell. Dead cells behave quite differently, for into their protoplasm, including the nucleus, the benzidine dyes diffuse rapidly, produc-

ing a uniform stain. Nothing is more striking than the way in which dead cells are stained in this manner. Liver cells selectively poisoned by chloroform[3] and renal cells killed by sublimate[4] both permit an immediate diffusion of the dye into them and resultant stain; and even the nerve cells, normally hostile to the entry of a single trace of the dye, receive it similarly when killed, an elegant example of which is furnished by the anterior horn cells in experimental poliomyelitis.[5] When we tap the cover slip over leucocytes swimming in trypan-blue, mechanical injury to the immediate subjacent cells invites instant entry of the dye.[5a]

It is evident then that into the dead cell a true diffusion takes place, but if diffusion be acting at all in the case of those living cells which react to the vital stain it is at least seriously hampered. There is in fact no reason for the identification of the vital stain with a diffusion phenomenon. It is significant that the cells which take the benzidine dyes are predominantly those endowed with powers of phagocytosis, a process long known to be operating in the case of particles from approximately 10 to 1 micron in size but it is not improbable that the colloidal particles of the benzidine dyes, whose dimensions must lie below a hundredth of such size, are received into the cell in an essentially similar way. Owing to their comparative minuteness, however, these particles are enabled to gain entry into many cells quite incapable of receiving larger ones. The fibroblast and mesothelial stains are examples of this. Yet in none of these cases does the cell, as it were, drink in the dye particles, as it does the freely diffusing ions of a salt solution. Nor indeed are the dye molecular-aggregates phagocytized in the usual acceptation of the term, for there is not merely a protoplasmic flow around a foreign body. Countless ultramicroscopic particles of the foreign body are let into the peripheral protoplasm and collected in the more central lying depots which we can at last recognize under the microscope as the dye granules.

This moving together or centralization of the dye in granules is not a reaction peculiar to the dyes, for an identical phenomenon is seen when colloidal silver is used, in which the particles have dimensions also considerably below the limits of ordinary microscopic vision and far below the dimensions of the intra-cellular granules in which they are later agminated.[6] Yet we have never hesitated to speak of the phagocytosis of silver aggregates in this later case.

We have to do then, in the case of cells which are stained vitally by these dyes, with a great host of elements scattered all over the body, serving in some special organs as the lining of blood and lymph vessels, but in the great interstitial tissue of the body without the vessels, equally abundant, cells whose primary function seems to be the engulfment of particles whose physical dimensions fall within certain limits. These scavenger cells, as it were, rid the blood and tissue juices of many kinds of useful and unuseful débris. How they do this may still be difficult to explain, but there seems no doubt but that their protoplasm in contrast to that of epithelial cells consists of a peculiar physical system.

[3] Experiments with Dr. Samuel J. Crowe, as yet unpublished.

[4] Gross, *Beitr. z. path. Anat.*, Bd. 51, p. 528, 1911.

[5] MacCurdy and Evans, *Berliner Med. Woch.*, 1912, No. 36.

[5a] Evans and Winternitz as yet unpublished.

[6] Bechold (*Zeitschr. f. chemie und Industrie der Kolloide*, 2 Jhrg., heft 1, 2) has determined that the aggregates of collargol-Heyden have a diameter of 20 $\mu\mu$. They consist of aggregates of metallic particles and particles of the schutz-kolloid together.

That useful and unuseful débris occurs normally in the body needs not, we take it, be defended, for the continual breaking down of some cellular elements of short life, the red blood corpuscles, for example, set free substances whose physical dimensions enable them to be engulfed by many of the cells which we have described (occurrence of blood pigment in liver, spleen, lymph glands and bone marrow) but that this reaction needs by no means be considered as one adapted for the engulfment of bodies of this class is proven conclusively by a number of observations made very recently by Ciaccio and others which go to show that fatty acids and lipoid substances are stored by the same cells. We are concerned then with cells of great physiological importance to the organism, cells whose action in this capacity, we believe, seems proven to be conditioned by physical and not chemical forces of response.

Herbert M. Evans,
Werner Schulemann

Comparative Registration Statistics[1]

One of the greatest difficulties encountered in the compilation of comparative university statistics is found in the apparent impossibility of securing uniformity. This difficulty is owing in large measure to two factors, one quantitative and the other qualitative. An illustration of the former is furnished by the fact that the student attending six weeks of summer session is recognized as a full unit, just as much as the student of an engineering school who annually puts in thirty-six hours a week for two half-years and several weeks in camp; and similarly a person engaged in secondary teaching who registers for a single late-afternoon or Saturday morning course counts as a full unit just as well as a candidate for the doctorate who spends his entire time at the university. Again, there are the students in so-called short courses, in agriculture, for example, who receive as much recognition as those who spend the entire year at the university. As a matter of fact, the most satisfactory solution along this line would be found in adopting a student-hour unit, but this, from the very nature of the case, would be an extremely complicated procedure. A simpler solution is reached by separating the summer session and short course students from those attending the entire year, and similarly by separating the full time from the partial time students. A difficulty would arise in connection with the point at which the line between these two groups is to be drawn, but this could readily be adjusted by agreement between the institutions involved. In this connection it might also be pointed out that owing to the fact that many secondary schools graduate classes in January as well as in June, several colleges and universities are admitting new students in February; they spend only half a year at the institution, but are counted as full units. On the other hand, the number of regular students enrolling for work in the summer session in order to reduce their time of residence or to make up conditions is constantly on the increase.

So far as the qualitative distinction is concerned, it must be borne in mind that the size of a university gives no more indication of its efficiency than the population of a country does of its degree of civilization, or the size of a city does of the morals and social welfare of its inhabitants. Comparative registration statistics, as they have been published by the writer from time to time in Science and elsewhere, have therefore little qualitative significance, inasmuch as such items as standards of admission and advancement, efficiency of instruction, equipment, and the like, are necessarily ignored in the comparison. So far as we are concerned in the present instance, standards of admission constitute perhaps the most significant item. No student should, in my opinion, be counted in the enrollment of a university, who has not offered graduation from a secondary school for admission. For-

[1] Paper presented at the annual meeting of the American Association of Collegiate Registrars, Richmond, Va., 1914.